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# A Nonflow Basis for the Vulnerability of the Subendocardium

CALVIN ENG, MD, SANGHO CHO, MD, STEPHEN M. FACTOR, MD, EDWARD S. KIRK, PHD

*Bronx, New York*

**The functional consequences of a transmural gradient of metabolism in the heart were studied in 19 dogs. The technique of retrograde blood flow diversion after coronary occlusion was used to deplete the ischemic myocardium of blood flow. Blood flow was uniformly and equally depleted in all layers, averaging 0.044 ml/min per g. With oxygen supply a controlled variable, transmural differences in metabolic demand can be addressed. In groups of dogs severe myocardial ischemia was induced for periods of 20 to 90 minutes. No necrosis was noted after 20 minutes of ischemia. Beginning at 30 minutes of blood flow depletion, necrosis progressed from the endocardium toward the epicardium in a "wave front" pattern. At 90 minutes of ischemia, approximately 70% of the area at risk was necrotic.**

**Thus, the relative vulnerability of the endocardium as compared with the epicardium is due to nonflow factors, and probably dictated by transmural differences in metabolic activity. It would appear that myocardial metabolism as compared with blood flow occupies a primary and overriding role during the first 20 minutes of ischemia. Furthermore, differences in transmural metabolism also dictate subendocardial vulnerability for ischemic periods greater than 20 minutes, irrespective of blood flow. The role of blood flow in these events may be to modulate the rate of the transmural wave front of progressing necrosis after 20 minutes of ischemia.**

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Ischemic damage to the myocardium preferentially affects the subendocardium. In dogs, Reimer et al. (1) noted that necrosis spreads in a wave front pattern from the endocardium toward the epicardium as the duration of coronary occlusion increases. This transmural pattern of myocardial injury has been confirmed in humans (2). The basis for this wave front pattern of necrosis and for subendocardial vulnerability is generally thought to be a transmural gradient of blood flow. With a severe coronary stenosis or after complete coronary occlusion in the dog, flow to the subendocardium is most compromised. A transmural gradient of extravascular resistance (3) accounts for this characteristic gradient of blood flow. However, several studies (4–10) have suggested a higher rate of metabolism in the subendocardium under physiologic conditions. Higher glycolytic enzyme activities (4), lower nicotinamide adenine dinucleotide (NAD)$^+$/nicotinamide adenine dinucleotide, reduced (NADH) ratios (5) and lower tissue oxygen tensions (6,7) have been reported for the subendocardium. In addition, several studies (8–10) have demonstrated a greater oxygen extraction in the endocardium. Thus, it is possible that the higher metabolic activity of the subendocardium may be the real basis for subendocardial vulnerability, irrespective of the blood supply gradient.

In order to assess the functional consequences of a possible transmural metabolic gradient, an experimental model is required that satisfies two conditions: 1) the transmural blood flow distribution must be uniform (controlled variable), and 2) ischemic conditions should be present. This study employed the technique of retrograde blood flow diversion to uniformly deplete the ischemic region of blood flow. When a coronary artery is cannulated and vented to atmosphere, blood flows retrograde from the cannula. This retrograde blood flow represents the collateral flow that would have entered the ischemic tissue if not diverted. Retrograde bleeding has been reported to deplete blood flow to all transmural layers (11,12). With blood flow uniformly depleted and thus a controlled variable, the functional consequences of the transmural metabolic gradient can be addressed.

From the Cardiovascular Research Laboratories, Division of Cardiology, Department of Medicine, Albert Einstein College of Medicine, Bronx, New York. This study was presented in part at the 55th Scientific Session of the American Heart Association, Dallas, Texas, 1982. It was supported in part by Grants HL-23171, HL-27219 and HL-29812 from the National Institutes of Health, Bethesda, Maryland and an Established Fellowship from the New York Heart Association, New York, New York. Dr. Eng is an established investigator for the New York Heart Association.

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Address for reprints: Calvin Eng, MD, Cardiovascular Research Laboratories, Albert Einstein College of Medicine, Forchheimer 715, 1300 Morris Park Avenue, Bronx, New York 10461.



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## Methods

**General surgical preparation.** Nineteen mongrel dogs of either sex weighing 20 to 34 kg were used in this study. The dogs were sedated with Innovar Vet (fentanyl, droperidol), 1 to 2 ml intravenously, before receiving methoxyflurane anesthesia (n = 5) or pentobarbital, 30 mg/kg, intravenous surgical anesthesia. The dogs were intubated and ventilated with an intermittent positive pressure respirator with 100% oxygen supplementation. The left chest was shaved and scrubbed with a betadine solution. Sterile surgical conditions were employed. A thoracotomy was performed at the fifth left intercostal space, and the heart was exposed in a pericardial cradle. Two polyvinyl catheters were passed into the left atrium through an incision in the left atrial appendage. One of the catheters was advanced into the left ventricular cavity to measure left ventricular pressure. The atrial catheter was used for microsphere injection. Polyvinyl catheters were also inserted into the femoral artery and vein at midthigh level. The femoral artery catheter was passed up into the thoracic aorta to measure systemic pressure, and for arterial blood reference withdrawal during microsphere injection.

The coronary anatomy was inspected for the presence of either a suitable moderate-sized first or second diagonal branch of the left anterior descending coronary artery or a comparably sized first or second marginal branch of the left circumflex coronary artery. The branch was dissected free near its junction with the main coronary artery. The main coronary artery was dissected free just proximal to the junction of the branch (Fig. 1). Heparin, 5,000 U, was given intravenously. The arterial branch was cannulated in a retrograde manner with a 15 or 18 gauge metal cannula. This cannula was connected to tubing that, when unclamped, allowed retrograde blood flow diversion after occlusion of the main coronary artery. An arterial side branch, rather than the main coronary artery, was cannulated because reperfusion and recovery for at least 24 hours are necessary to histologically delineate necrosis. Cannulation of the main artery would have required an arterial repair, which has inherent problems. Thus, only the cannulated side branch was ultimately sacrificed, and the subsequent small area of necrosis was not analyzed in the results (Fig. 1).

**Experimental protocol.** The experimental protocol was as follows: 1) A set of microspheres was injected before any intervention for calculation of microsphere loss (13,14). The microspheres used were 9 $\mu$ in diameter (3M Company) and labeled with either iodine-125, cerium-141, chromium-51, strontium-85 or scandium-46. Five million microspheres were injected into the left atrium. Withdrawal of reference arterial blood was begun just before injection and continued for 1 minute at a rate of 19.4 ml/min. 2) A temporary coronary occlusion was performed with a silk ligature snare on the main artery of interest (Fig. 1). The tubing connected to the cannulated arterial branch was opened to atmosphere at the level of the right atrium, and a minute collection of retrograde blood flow was measured (control retrograde blood flow). The tubing was then clamped and flow restored to the coronary artery by release of the ligature. Five minutes was allowed for recovery. 3) The coronary artery was reoccluded with the snare, and retrograde bleeding commenced again by opening the tubing to atmosphere. This occlusion with retrograde blood flow diversion was maintained for a period of 20 to 90 minutes in an individual experiment. Intravenous lidocaine (20 to 40 mg) was given as required for arrhythmias. 4) Ten minutes before reperfusion, a second set of microspheres was injected to assess the transmural distribution of blood flow during retrograde bleeding ischemia, and a second timed collection of retrograde blood flow was determined. 5) The retrograde bleeding was terminated by clamping the tubing and the major coronary vessel was reperfused by removing the snare. 6) The cannulated arterial side branch was then permanently ligated and the cannula removed. 7) All catheters were removed, the chest was closed and the lungs were reexpanded. 8) Antibiotic and analgesic agents were given, and the dogs were allowed to recover for a period of 24 to 72 hours. Antibiotic agents were given daily and analgesic agents were given as required.

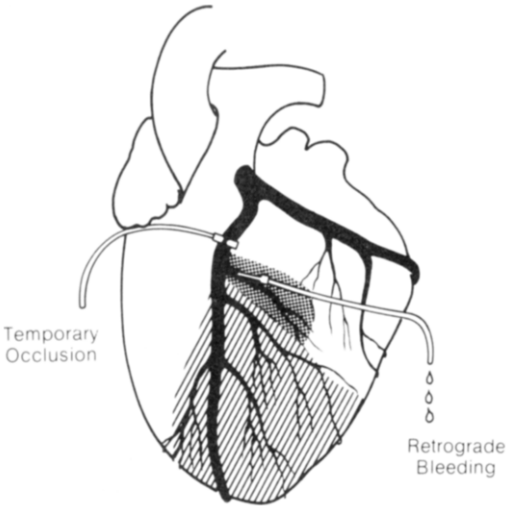


**Figure 1.** Experimental preparation. A small diagonal or marginal branch of either the left anterior descending or left circumflex coronary artery was cannulated in a retrograde manner. A temporary snare was placed on the major coronary artery just proximal to the junction of the branch. With the snare occluded, the tubing connected to the cannulated branch was opened to atmosphere. Blood flows retrograde from the cannula, depleting the myocardium of collateral flow and producing a uniform transmural depletion of blood flow. The arterial branch was sacrificed, and the region it supplies (**dotted area**) was not included in the analysis. The **hatched area** represents the ischemic region that was analyzed for blood flow and histologic features.

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9) After the recovery period, the dogs were again anesthetized with pentobarbital (30 mg/kg, intravenously), intubated and ventilated. A thoracotomy was performed to expose the heart. The site of temporary occlusion on the coronary artery was identified and directly cannulated with a 13 gauge metal cannula. In all dogs, free retrograde bleeding was confirmed. Timed volume collections of retrograde blood flow at sacrifice were measured in eight dogs. 10) A lethal dose of potassium chloride was given and the heart was removed. Microfil was injected into the coronary cannula to delineate the area at risk (15,16) and the heart was fixed in formalin.

**Blood flow and histologic analysis.** The hearts were sliced in breadloaf fashion from apex to base giving approximately six sections. The apical and basal portions of the heart were not used in the analysis. One or two alternating sections were sent for histologic analysis. Two sections were used to determine myocardial blood flow on four to eight transmural samples (two to four each from the ischemic zone and the normal zone). Each transmural sample was divided into endocardial, midwall and epicardial segments. The tissue samples and the reference arterial blood were subjected to gamma spectrometry (Searle Analytic model 1085 with a Nuclear Data ND-60 pulse height analyzer). Calculations and corrections for crossover and background were performed on a Wang 2200S computer. Cardiac output was determined from the reference arterial blood. Calculations of microsphere loss in the ischemic region were determined by the difference in blood flow between the normal and ischemic zones during the initial control microsphere injection. Microsphere loss was calculated for each transmural layer of the ischemic zone and expressed as percent loss. Myocardial blood flow is presented as originally measured and after correction for microsphere loss (17).

*The tissue for histologic analysis was embedded in paraffin.* The ischemic zone as defined by the Microfil was divided into two or three sectors. Five micron thick sections of these sectors were stained with hematoxylin-eosin. The entire risk region as outlined by the Microfil and the regions of myocardial necrosis were quantitated using point counting methods as previously described from this laboratory (15,16). The extent of necrosis is presented as a percent of the region at risk. The small region of necrosis due to the sacrificed arterial side branch was not filled with Microfil, and was readily distinguished from the regions of interest.

**Statistical analysis.** Summary data are presented as mean ± SEM. Student's paired *t* test was used to compare the hemodynamic variables. Analysis of variance was used for comparisons of flows and microsphere loss in the three transmural regions. The Scheffé multiple comparison test was used after a significant F value was obtained. A probability (p) value of less than 0.05 was considered a significant difference.

## Results

Direct current cardioversion was required in eight dogs during coronary occlusion and in eight dogs after reperfusion. One dog had ventricular fibrillation before and after reperfusion. Four dogs required no cardioversion.

**Hemodynamic variables.** Hemodynamic values before coronary occlusion compared with those 10 minutes before reperfusion were as follows (n = 19): heart rate = 128 ± 6 versus 133 ± 6 beats/min (p = NS); mean aortic pressure = 100 ± 4 versus 96 ± 4 mm Hg (p = NS); left ventricular diastolic pressure = 6.7 ± 0.5 versus 11.9 ± 1.2 mm Hg ($p < 0.0025$); and cardiac output = 4,121 ± 227 versus 3,330 ± 221 ml/min ($p < 0.001$). Retrograde blood flow was not quantitated in all experiments and was as follows: 3.7 ± 0.7 (control, n = 17), 3.1 ± 0.7 (10 minutes before reperfusion, n = 13) and 4.3 ± 0.7 ml/min (at sacrifice, n = 8). These values were not statistically different from each other (analysis of variance).

**Myocardial blood flow.** Control myocardial blood flow to the normal zone was as follows: 0.823 ± 0.078 (endocardium), 0.867 ± 0.084 (midwall) and 0.787 ± 0.078 (epicardium) ml/min per g. During coronary occlusion and retrograde bleeding, myocardial blood flow to the normal zone was as follows: 0.857 ± 0.068 (endocardium), 0.884 ± 0.074 (midwall) and 0.777 ± 0.070 (epicardium) ml/min per g. The epicardial layer had slightly less flow than the midwall or endocardium ($p < 0.025$). In the ischemic zone, the calculated percent loss of microspheres for each layer was: 30.8 ± 5.7% (endocardium), 25.7 ± 4.9% (midwall) and 15.4 ± 4.8% (epicardium). The microsphere loss was significantly less in the epicardium as compared with the endocardium ($p < 0.01$) and the midwall ($p = 0.08$). Because the extent of microsphere loss parallels the extent of necrosis (13), these results independently confirm that the subendocardium was most vulnerable to necrosis, and that there is a transmural gradient of injury.

*Table 1 summarizes the blood flow results in the ischemic tissue during retrograde bleeding.* Results uncorrected and corrected for microsphere loss are presented and reveal a uniform transmural depletion of blood flow during retro-

**Table 1.** Myocardial Blood Flow in the Ischemic Area During Coronary Occlusion and Retrograde Bleeding in 19 Dogs

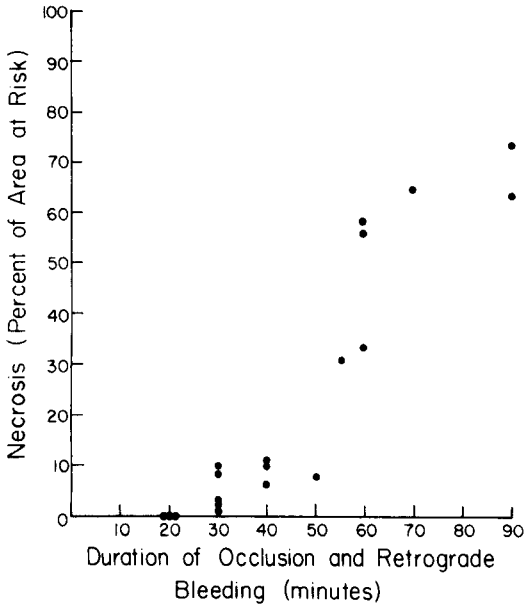
| | Myocardial Blood Flow (ml/min per g) | |
|---|---|---|
| | Uncorrected Value | Corrected Value* |
| Epicardium | 0.029 ± 0.005 | 0.036 ± 0.008 |
| Midwall | 0.020 ± 0.006 | 0.037 ± 0.012 |
| Endocardium | 0.021 ± 0.006 | 0.060 ± 0.032 |

*Value corrected for microsphere loss. Data are presented as mean ± SEM. Transmural flow values are not significantly different on a 3 × 2 analysis of variance with repeated measures.

grade bleeding. The blood flow values did not differ transmurally, and microsphere correction did not significantly change the results (3 × 2 analysis of variance with repeated measures, n = 19). Although after correction the subendocardium had a slightly greater flow value (0.060 ± 0.032 ml/min per g), it was not different from that in other layers. A slightly higher flow value in the endocardium would only further emphasize the observation that the endocardium was most vulnerable to infarction. These flow values during retrograde bleeding are an order of magnitude lower than those usually found during typical coronary occlusion.

**Wave front pattern of necrosis.** The histologic quantitation of necrosis for the various periods of uniform blood flow depletion is illustrated in Figure 2. Eleven dogs had left anterior descending coronary artery occlusion and eight dogs had circumflex coronary artery occlusion. Necrosis as a percent of the risk region was as follows (left circumflex artery occlusions are identified as LCx): 1) 20 minutes (n = 3): 0%(LCx), 0%(LCx), 0%(LCx); 2) 30 minutes (n = 5): 1.3%, 2.7%, 3.8%(LCx), 8.5%, 9.9%; 3) 40 minutes (n = 3): 6.3%(LCx), 9.8%(LCx), 10.7%; 4) 50 minutes (n = 1): 8.2%(LCx); 5) 55 minutes (n = 1): 31%(LCx); 6) 60 minutes (n = 3): 32.1%, 54.4%, 58.4%; 7) 75 minutes (n = 1): 64.4%; 8) 90 minutes (n = 2): 64%, 72.8%. The interval between 40 and 60 minutes of ischemia brackets a rapid period of necrosis progression from 10 to 60%. No necrosis was noted in the three animals depleted of blood flow for 20 minutes. Furthermore, necrosis, when present, was always localized to the endocardial layer with extension toward the epicardium for longer periods of ischemia. Thus, necrosis conformed to a wave front pattern with spread from the endocardium toward the epicardium (1) despite a uniform depletion of blood flow.

**Figure 2.** Myocardial necrosis in relation to the duration of coronary occlusion and blood flow depletion in 19 dogs (see text).




## Discussion

**Subendocardial vulnerability.** Ischemic damage to the myocardium preferentially affects the subendocardium. This phenomenon is borne out in clinical situations such as aortic valve disease, shock and, commonly, in nontransmural myocardial infarction due to coronary artery disease. Reimer et al. (1) observed that after 40 minutes of coronary occlusion in the dog, necrosis was confined to the subendocardium. However, with longer periods of occlusion, the necrosis progressed transmurally as a wave front toward the epicardium. Traditionally, subendocardial vulnerability has been ascribed to the characteristic transmural blood flow gradient that is observed under conditions of coronary hypoperfusion. The greater extravascular compressive forces on endocardial vessels result in a greater flow decrement in the endocardium as compared with the outer layers. On the other side of the supply/demand relation, several studies (4–10) have indicated that the metabolic activity of the endocardium is greater than that of the outer layers under physiologic conditions. However, the functional significance of these studies is unclear with regard to subendocardial vulnerability during ischemia. It is possible that, although the endocardium has a greater metabolic activity under physiologic conditions, this relative "disadvantage" of the endocardium compared with the epicardium can be rapidly dissipated within a short period of time during ischemia. In order for the metabolic gradient to be significant, the gradient must be demonstrated to persist under ischemic conditions and have a time course consistent with the production of necrosis. The blood flow gradient has been documented to persist through the onset of necrosis and beyond. Thus, the presumed metabolic disadvantage of the endocardium under normal physiologic conditions cannot be readily extrapolated to ischemic conditions.

**Blood supply as a controlled variable.** Dunn and Griggs (18) stopped coronary flow by occluding all coronary arteries. Biopsy samples of the myocardium revealed higher lactate levels in the endocardium than in the epicardium at 15 and 30 seconds of ischemia. Inducing ventricular fibrillation at the onset of no flow ischemia did not result in a transmural gradient of lactate, suggesting the importance of wall stress for the metabolic gradient. Rivas et al. (19) explored the relation between collateral blood flow and the extent of myocardial necrosis after a coronary occlusion. One of their findings was that, for a given level of collateral blood flow, the endocardium had a disproportionately greater extent of infarction. These results are compatible with a greater subendocardial vulnerability. However, a redistribution of collateral flow toward the epicardium (20) or a greater loss of microspheres from the endocardium (13,17),

or both, could also explain these results. Fujiwara et al. (21) determined the transmural effects of coronary occlusion in pigs. The collateral flow in pigs was minimal, and averaged less than 0.05 ml/min per g, with no transmural gradient of collateral flow. Light and electron microscopic criteria for myocardial cell damage were used in this nonrecovery study. A greater injury score was noted for the endocardial layer at 20 minutes of ischemia. By 120 minutes of ischemia, the transmural extent of cell injury was uniform, suggesting that the wave front had terminated and the maximal extent of injury was achieved by this period. In this important and convincing study, the authors suggested that nonflow factors such as wall stress or metabolism, or both, dictated the transmural results. Lowe et al. (22) determined the time course of adenosine triphosphate (ATP) depletion in globally ischemic excised hearts. ATP depleted more rapidly in the endocardium as compared with the epicardium, and this difference persisted for more than 1 hour. In this preparation, both blood flow and wall stress were abolished, so that the greater rate of ATP depletion in the endocardium presumably represents an intrinsic higher metabolic rate in the endocardium.

**The primacy of metabolism during ischemia.** In our study, we employed the technique of retrograde blood flow diversion after coronary occlusion to produce a uniform transmural blood flow depletion. Myocardial blood flow during retrograde blood flow averaged 0.044 ml/min per g, after correction for microsphere loss. This value of blood flow is one order of magnitude lower than flow values usually observed after typical coronary occlusion in the dog. Thus, the magnitude of the flow depletion was very severe. Despite the severity of flow depletion, necrosis only began with occlusions of 30 minutes or longer. There was no evidence of necrosis after ischemia of 20 minutes' duration. This time course of the onset of necrosis is quite similar to that of typical coronary occlusion in the dog (23). If blood flow directly modulates cell viability, then it would be expected that with such low blood flow values during retrograde bleeding, necrosis would occur very early and that the 20 minute period of reversibility established for typical coronary occlusion would not be applicable. The similarity of time course suggests that metabolism is the *sole* determinant of cell viability during the first 20 minutes of ischemia. The actual level of blood flow appears to have no influence during this initial 20 minute period. Thus, modifications of blood flow below a presumed critical value would not alter the inevitable metabolic determinants of cell viability. However, our study cannot address this issue directly without a comparable series of typical occlusion experiments. It also should be noted that the extent of necrosis at 40 minutes in our study (about 10%) is not as great as the extent of necrosis noted in other studies (1,16) for reasons that are unclear.

*For ischemic periods greater than 20 minutes, metabolic factors also appear to predominate.* The wave front pattern of necrosis is independent of blood flow in our study and others (21,22). However, the role of blood flow may be twofold: 1) It is a permissive factor. Below a presumed critical flow value, the metabolic determinants are allowed to be expressed fully. 2) It is likely that the level of blood flow may modulate the *rate* of transmural progression of the wave front phenomenon. In Figure 2, there appears to be an explosive increase in the extent of necrosis between a narrow 50 to 60 minute period of ischemia. Furthermore, in our study approximately 70% of the region at risk was necrotic at 90 minutes of severe blood flow depletion. In the study of Reimer et al. (1), 6 hours of ischemia (with collateral flow) was required to attain 70% necrosis.

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